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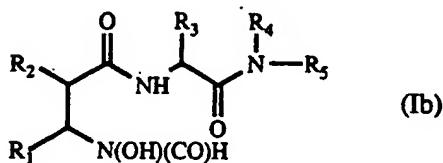
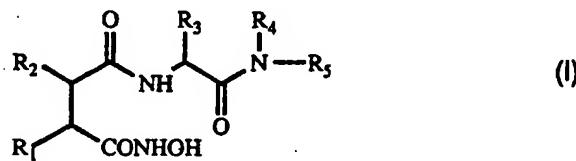
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(54) Title: INHIBITION OF TNF PRODUCTION



(57) Abstract

Certain hydroxamic acid derivatives, previously known in the art as inhibitors of matrix metalloproteinases such as collagenase are capable of inhibiting the production of tumour necrosis factor (TNF) by cells, and thus are useful in the management of diseases or conditions mediated by overproduction of, or over-responsiveness to, TNF. The compounds in question are known in the art from the following patent publications: US 4599361, EP-A-0236872, EP-A-0274453, WO 90/05716, WO 90/05719, WO 91/02716, EP-A-0489577, EP-A-0489579, EP-A-0497192, WO 92/13831, WO 92/22523, WO 93/09090, and WO 93/09097. They have general formula (I) or (Ib) wherein substituents R₁-R₅ may vary widely according to the disclosures of those patent publications.

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INHIBITION OF TNF PRODUCTION

This invention relates to a new pharmaceutical and veterinary use of certain hydroxamic acid derivatives, previously known in the art as inhibitors of matrix metalloproteinases such as collagenase. In accordance with the invention the known compounds have been found to be capable of inhibiting the production of tumour necrosis factor (TNF) by cells, and thus to be useful in the management of diseases or conditions mediated by overproduction of, or over-responsiveness to, TNF.

Background to the Invention

TNF

TNF is a cytokine which is produced initially as a cell-associated 28kD precursor. It is released as an active, 17kD form (Jue, D-M et al., (1990) Biochemistry 29:8371-8377), which can mediate a large number of deleterious effects *in vivo*. When administered to animals or humans it causes inflammation, fever, cardiovascular effects, haemorrhage, coagulation and acute phase responses, similar to those seen during acute infections and shock states. Chronic administration can also cause cachexia and anorexia. Accumulation of excessive TNF can be lethal.

There is considerable evidence from animal model studies that blocking the effects of TNF with specific antibodies can be beneficial in acute infections, shock states, graft versus host reactions and autoimmune disease. TNF is also an autocrine growth factor for some myelomas and lymphomas and can act to inhibit normal haematopoiesis in patients with these tumours.

Preventing the production or action of TNF is, therefore, predicted to be a potent therapeutic strategy for many inflammatory, infectious, immunological or malignant diseases.

It has been shown that the effects of TNF are mediated by two peptides, TNF α and TNF β . Although these peptides have only 30% homology with each other, they activate the same receptors and are encoded by immediately adjacent genes. As

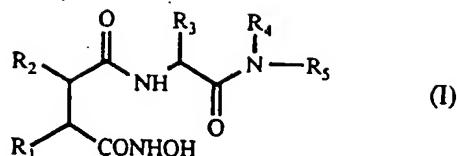
used herein, the term tumour necrosis factor or TNF therefore means tumour necrosis factor α and peptides having a high degree of sequence homology with, and substantially similar physiological effects to, TNF α , for example TNF β .

MMP Inhibitors

It is known that metalloproteinases such as collagenase, stromelysin and gelatinase (known as "matrix metalloproteinases", and herein referred to as MMPs) are involved in connective tissue breakdown. The known hydroxamic acid-based MMP inhibitors with which this invention is concerned are those forming part of the state of the art by virtue of any of the following patent publications:

US 4599361	("Searle")
EP-A-0236872	("Roche 1")
EP-A-0274453	("Bellon")
WO 90/05716	(British Bio-technology) ("BBL1")
WO 90/05719	(British Bio-technology) ("BBL2")
WO 91/02716	(British Bio-technology) ("BBL3")
EP-A-0489577	("Celltech 1")
EP-A-0489579	("Celltech 2")
EP-A-0497192	("Roche 2")
WO 92/13831	(British Bio-technology) ("BBL4")
WO 92/22523	(Research Corporation Technologies) ("RCT")
WO 93/09090	("Yamanouchi")
WO 93/09097	("Sankyo")

The disclosure of each of those publications is hereby incorporated by reference, and the reader is referred thereto for details of the structures of the compounds disclosed and methods for their preparation. The MMP inhibiting hydroxamic acid derivatives disclosed therein can be regarded as having the following basic structure (I) :

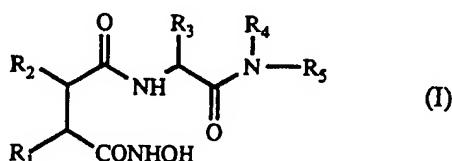


wherein the five substituents R₁ - R₅ may vary according to the detailed disclosure of each publication. However, neither the compounds *per se* nor the method of their preparation form part of this invention. Rather, the invention arises from the finding by the present inventors that representative members of the compound classes disclosed in each of those publications have the property of inhibiting release of TNF from cells, and the realisation that such activity is widespread amongst the disclosed compounds.

General Composite Structural Definition of Known MMP Inhibitors

Without prejudice to the true disclosures of the patent publications listed above, the following general composite structural definition of the disclosed compounds is offered as a guide. It has been compiled from the structural disclosures of the publications, but where further information is required concerning the identity of the compounds with which the present invention is concerned, the reader must consult the publication in question. Furthermore, where conflict arises between the disclosure of any of the patent publications listed above and the general composite structural definition set out below, the former prevails:

The aforesaid composite structural definition is of compounds of formula (I) or (Ib)



wherein the five substituents R₁ - R₅ may vary as follows:

- R₁ (Searle) hydrogen, C₁-C₆ alkyl, phenyl, phenyl(C₁-C₆ alkyl);
(Roche 1) hydrogen, NH₂, OH, SH, C₁-C₆ alkyl, C₁-C₆ alkoxy, C₁-C₆ alkylamino, C₁-C₆ alkylthio or aryl(C₁-C₆ alkyl) group or amino-C₁-C₆ alkyl, hydroxy-C₁-C₆ alkyl, mercapto-C₁-C₆ alkyl or carboxy-C₁-C₆ alkyl where the amino-, hydroxy-, mercapto or carboxyl group can be protected, the amino group acylated or the carboxyl group amidated;
(Bellon) hydrogen;
(BBL1) hydrogen, C₁-C₆ alkyl, C₂-C₆ alkenyl, phenyl, phenyl(C₁-C₆ alkyl), or a group BSO_nCH₂- wherein n is 0, 1 or 2 and B is hydrogen or a (C₁-C₆) alkyl, phenyl, substituted phenyl, phenyl(C₁-C₆)alkyl, or heterocyclyl group;
(BBL2) a group BSO_nA- wherein n is 0, 1 or 2 and B is hydrogen or an alkyl, phenyl, substituted phenyl, phenylalkyl, heterocyclyl, alkylcarbonyl, phenacyl or substituted phenacyl group, and A represents a hydrocarbon chain optionally substituted with one or more alkyl, phenyl, or substituted phenyl groups;
(BBL3) hydrogen or a (C₁-C₆) alkyl, (C₂-C₆) alkenyl, phenyl, phenyl(C₁-C₆)alkyl, (C₁-C₆)alkylthiomethyl, phenylthiomethyl, substituted phenylthiomethyl, phenyl(C₁-C₆)alkylthiomethyl or heterocyclylthiomethyl group;
(Celltech 1) optionally substituted alkyl, alkenyl, aryl, aralkyl, heteroaralkyl, or heteroarylthioalkyl;
(Celltech 2) optionally substituted alkyl, alkenyl, aryl, aralkyl, heteroaralkyl, or heteroarylthioalkyl;
(Roche 2) hydrogen, amino, protected amino, acylamino, or lower alkyl optionally substituted by aryl, hydroxy, protected hydroxy, amino, protected amino, acylamino, maleimido, succinimido, naphthalimido, 2,3-dihydro-1,3-dioxo-1H-benz[d,e]isoquinol-2-yl, carboxy, protected carboxy, carbamoyl, mono(lower alkyl)carbamoyl, di(lower alkyl)carbamoyl, di(lower alkyl)amino, carboxy-lower alkanoylamino, pyrrolidino or morpholino;
(BBL4) hydrogen (C₁-C₆) alkyl, phenyl, substituted phenyl, phenyl(C₁-C₆)alkyl, or heterocyclyl; or R₁ represents ASO_nR₇ wherein A represents a (C₁-C₆) hydrocarbon chain, optionally substituted with one or more (C₁-C₆) alkyl, phenyl or substituted phenyl groups and n=0, 1 or 2; R₇ is (C₁-

C_6 alkyl, phenyl, substituted phenyl, phenyl (C_1 - C_6) alkyl, heterocyclyl, (C_1 - C_6)alkyl, (C_1 - C_6)acyl, thienyl or phenacyl;
("RCT") hydrogen, lower alkyl, aryl, or aryl lower alkyl;
(Yamanouchi) lower alkyl which may be substituted by a substituent selected from mercapto, loweralkylthio, arylthio and lower acylthio;
(Sankyo) hydrogen;

- R_2 (Searle) hydrogen, phenyl, phenyl(C_1 - C_6 alkyl), cycloalkyl, cycloalkyl(C_1 - C_6 alkyl);
(Roche 1) C_2 - C_5 alkyl;
(Bellon) isobutyl;
(BBL1) hydrogen atom or an (C_1 - C_6)alkyl, (C_2 - C_6)alkenyl, phenyl(C_1 - C_6)alkyl, cycloalkyl (C_1 - C_6)alkyl or cycloalkenyl (C_1 - C_6)alkyl group;
(BBL2) a hydrogen atom or an alkyl, alkenyl, phenylalkyl, cycloalkylalkyl or cycloalkenylalkyl group;
(BBL3) a hydrogen atom or an (C_1 - C_6)alkyl, (C_2 - C_6)alkenyl, phenyl(C_1 - C_6)alkyl, cycloalkyl (C_1 - C_6)alkyl or cycloalkenyl (C_1 - C_6)alkyl group;
(Celltech 1) optionally substituted alkyl, alkenyl, cycloalkyl, aryl, aralkyl, aralkoxy, or aralkylthio group, or an amino, substituted amino, carboxyl, or esterified carboxyl group;
(Celltech 2) optionally substituted phenylethyl, phenylpropyl or phenylbutyl group;
(Roche 2) isobutyl;
(BBL4) hydrogen or an (C_1 - C_6)alkyl, (C_2 - C_6)alkenyl, phenyl(C_1 - C_6)alkyl, or cycloalkyl (C_1 - C_6)alkyl group;
("RCT") hydrogen, lower alkyl, aryl, or aryl lower alkyl;
(Yamanouchi) a group -Y-X-Z wherein Y is a single bond or lower alkylene, X is O or S, and Z is lower alkyl;
(Sankyo) hydrogen, alkyl or aralkyl;
- R_3 (Searle) C_1 - C_6 alkyl, benzyl, benzyloxybenzyl, (C_1 - C_6 alkoxy)benzyl, benzyloxy(C_1 - C_6 alkyl);
(Roche 1) the characterising group of a natural α amino acid, which may

be protected if functional groups are present, eg acylation of amino groups and amidation of carboxylgroups, with the provision that R₃ is not H or methyl;

(Bellon) isopropyl, 4-aminobutyl, n-butyl, 2-methylmercaptoethyl;

(BBL1) an amino acid residue with R or S stereochemistry or a (C₁-C₆)alkyl, benzyl, (C₁-C₆)alkoxy benzyl or benzyloxy (C₁-C₆)alkyl group;

(BBL2) an amino acid residue with R or S stereochemistry or a (C₁-C₆)alkyl, benzyl, (C₁-C₆)alkoxy benzyl or benzyloxy (C₁-C₆)alkyl group;

(BBL3) an amino acid residue with R or S stereochemistry or a (C₁-C₆)alkyl, benzyl, (C₁-C₆)alkoxy benzyl, benzyloxy (C₁-C₆)alkyl or benzyloxybenzyl group;

(Celltech 1) a group -[Alk]_nR⁶ where Alk is an alkyl or alkenyl group optionally interrupted by one or more -O-, or -S- atoms or -N(R⁷)-groups [where R⁷ is a hydrogen atom or a C₁-C₆ alkyl group], n is zero or an integer 1, and R⁶ is an optionally substituted cycloalkyl or cycloalkenyl group;

(Celltech 2) optionally substituted alkyl or alkenyl group optionally interrupted by one or more -O-, or -S- atoms or -N(R⁷)-groups [where R⁷ is a hydrogen atom or a C₁-C₆ alkyl group];

(Roche 2) tert-butyl;

(BBL4) a benzyl group with one or two substituents in the phenyl ring selected from hydrogen, halogen, cyano amino, amino (C₁-C₆)alkyl, amino di (C₁-C₆)alkyl, amino (C₁-C₆)alkylacyl, aminophenacyl, amino(substituted)phenacyl, amino acid or derivative thereof, hydroxy, oxy (C₁-C₆)alkyl, oxyacyl, formyl, carboxylic acid, carboxamide, carboxy (C₁-C₆)alkylamide, carboxyphenylamide, carboxy (C₁-C₆)alkyl, hydroxy (C₁-C₆)alkyl, (C₁-C₆)alkyloxy (C₁-C₆)alkyl or acyloxy (C₁-C₆)alkyl, (C₁-C₆)alkylcarboxylic acid, or (C₁-C₆)alkylcarboxy (C₁-C₆)alkyl, amino(C₁-C₆)alkylacyl carboxylic acid or amino(C₁-C₆)alkylcarboxylate; or a benzyl group with one substituent in the phenyl ring selected from groups of formula -OCH₂CN, -OCH₂COR⁸ and -OCH₂CH₂OR⁹, where R⁸ is hydroxyl, (C₁-C₆)oxyalkyl, (C₁-C₆)oxyalkylphenyl, amino, (C₁-C₆)aminoalkyl, (C₁-C₆)aminodialkyl, (C₁-C₆)aminoalkylphenyl, an amino acid or derivative

thereof; and R⁹ is (C₁-C₆)alkyl, (C₁-C₆)alkylphenyl, phenyl, substituted phenyl (C₁-C₆)alkylacyl or phenacyl;
("RCT") aryl lower alkyl, or heterocyclic lower alkyl, either being unsubstituted or mono- or di-substituted with fluoro, bromo, chloro, halo, nitro, carboxy, lower carbalkoxy, cyano, lower alkanoyl, trifluoromethyl lower alkyl, hydroxy, lower alkoxy, formyl, amino, lower alkyl amino, di-lower alkyl amino, mercapto, lower alkylthio, or mercapto lower alkyl;
(Yamanouchi) 4-(lower alkyl)phenylmethyl;
(Sankyo) a group -CH₂-CH₂-CH₂-NH- where the right hand bond is formed with the N atom adjacent the C atom carrying R₃, thereby replacing the H atom shown on that C atom in formula (I);

- R₄ (Searle) C₁-C₆ alkyl;
(Roche 1) a group -CHBD wherein B is H or methyl and D is H or C₁-C₆ alkyl, C₁-C₆ alkoxy-C₁-C₆ alkyl, di(C₁-C₆ alkoxy)methyl, carboxy, C₁-C₆ alkylcarbonyl, C₁-C₆ alkoxy carbonyl, arylmethoxycarbonyl, C₁-C₆ alkylaminocarbonyl or arylaminocarbonyl, or B and D together form a trimethylene group; or R₃ and B together form a group (CH₂)_n where n is an integer from 4-11;
(Bellon) hydrogen, phenyl(C₁-C₂ alkyl), or trifluoromethylphenyl(C₁-C₂ alkyl);
(BBL1) a group -(CH₂)_nA wherein n is an integer from 1 to 6 and A represents the group -NH₂, a substituted acyclic amine or a heterocyclic base;
(BBL2) a hydrogen atom or a (C₁-C₆) alkyl group;
(BBL3) a group (CH₂)_nA wherein n is an integer from 1 to 6 and A represents a hydroxy, (C₁-C₆) alkoxy, (C₂-C₇) acyloxy, (C₁-C₆) alkylthio, phenylthio, (C₂-C₇) acylamino or N-pyrrolidone group;
(Celltech 1) a hydrogen atom, an optionally substituted straight or branched alkyl group, optionally interrupted by one or more -O- or -S-atoms or -N(R⁷)-groups [where R⁷ is a hydrogen atom or a C₁-C₆ alkyl group], or aminocarbonyloxy groups; or R⁴ and R⁵ together with the nitrogen

atom to which they are attached may form an optionally substituted C₃-C₆ cyclic amino group optionally possessing one or more other heteroatoms selected from -O- or -S- or -N(R⁷)-groups [where R⁷ is a hydrogen atom or a C₁-C₆ alkyl group];

(Celltech 2) a hydrogen atom, an optionally substituted straight or branched alkyl group, optionally interrupted by one or more -O- or -S-atoms or -N(R⁷)-groups [where R⁷ is a hydrogen atom or a C₁-C₆ alkyl group], or aminocarbonyloxy groups; or R⁴ and R⁵ together with the nitrogen atom to which they are attached may form an optionally substituted C₃-C₆ cyclic amino group optionally possessing one or more other heteroatoms selected from -O- or -S- or -N(R⁷)-groups [where R⁷ is a hydrogen atom or a C₁-C₆ alkyl group];

(Roche 2) hydrogen or lower alkyl optionally substituted by aryl, amino, protected amino, di(lower alkyl)amino, guanidino, carboxyl, protected carboxyl, carbamoyl, mono(lower alkyl)carbamoyl, di(lower alkyl)carbamoyl, di(lower alkyl)phosphinyl, dihydroxyphosphinyl, pyrrolidino, piperidino or morpholino;

(BBL4) hydrogen or a (C₁-C₆)alkyl, or phenyl(C₁-C₆)alkyl group; ("RCT") a group -XD wherein X is a bond, lower alkylene, or a group -(CAB)_m-C(O)-N(Z)-, or -(CAB)_m-CH₂O-, or -(CAB)_m-C(O)O- wherein A is hydrogen, methyl or ethyl, and B and Z are independently hydrogen or lower alkyl, and m is 1, 2 or 3 with the proviso that when X is a chemical bond then the group R₃ (see above for RCT definition of R₃) is not unsubstituted benzyl or benzyl monosubstituted with hydroxy or lower alkoxy, and the further proviso that when X is a group -(CAB)_m-C(O)-N(Z)- wherein m is 1 and A, B and Z are as defined above, then the group R₃ (see above for RCT definition of R₃) is not indole or imidazole or unsubstituted benzyl or benzyl substituted with hydroxy or lower alkoxy;

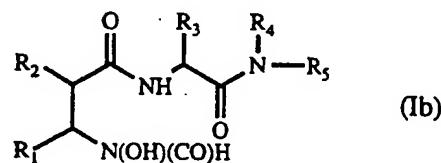
(Yamanouchi) lower alkyl;

(Sankyo) hydrogen, alkyl, alkoxy, or (when R⁵ is hydrogen) -CH(R⁶)COR⁷ where R⁶ is hydrogen or alkyl and R⁷ is alkyl, or -CH(R⁶)COOR⁸ where R⁶ is hydrogen or alkyl and R⁸ is alkyl, or -CH(R⁶)CONR⁹R¹⁰ where R⁹ and R¹⁰ are each alkyl, or R⁴ taken together with R⁵ and the nitrogen atom to which

they are attached form a heterocyclic group;

- R₅ (Searle) hydrogen;
- (Roche 1) H or methyl;
- (Bellon) hydrogen;
- (BBL1) a hydrogen atom or a methyl group;
- (BBL2) a hydrogen atom or a methyl group;
- (BBL3) a hydrogen atom or a methyl group;
- (Celltech 1) a hydrogen atom, an optionally substituted straight or branched alkyl group, optionally interrupted by one or more -O- or -S-atoms or -N(R⁷)-groups [where R⁷ is a hydrogen atom or a C₁-C₆ alkyl group], or aminocarbonyloxy groups;
- (Celltech 2) a hydrogen atom, an optionally substituted straight or branched alkyl group, optionally interrupted by one or more -O- or -S-atoms or -N(R⁷)-groups [where R⁷ is a hydrogen atom or a C₁-C₆ alkyl group], or aminocarbonyloxy groups;
- (Roche 2) hydrogen;
- (BBL4) hydrogen or methyl;
- ("RCT") hydrogen or lower alkyl;
- (Yamanouchi) hydrogen;
- (Sankyo) hydrogen or alkyl.

In addition, the publication (Roche 1) discloses "reverse hydroxamic acid" MMP inhibitors of formula (Ib)



wherein the substituents R₁ - R₂ are the "Roche 2 substituents defined above in relation to formula (I).

Salts of such known MMP inhibitors include physiologically acceptable acid addition salts for example hydrochlorides, hydrobromides, sulphates, methane sulphonates, p-toluenesulphonates, phosphates, acetates, citrates, succinates, lactates, tartrates, fumarates and maleates. Salts may also be formed with bases, for example sodium, potassium, magnesium, and calcium salts.

There are several chiral centres in such known MMP inhibitors because of the presence of asymmetric carbon atoms. The presence of several asymmetric carbon atoms gives rise to a number of diastereomers with R or S stereochemistry at each chiral centre. General formula (I), and (unless specified otherwise) all other formulae in this specification are to be understood to include all such stereoisomers and mixtures (for example racemic mixtures) thereof.

The preferred stereochemistry is in general as follows:

C atom adjacent the -CONHOH moiety - S,

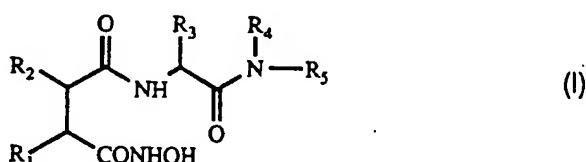
C atom adjacent the R₂ group - R,

C atom adjacent the R₃ group - S,

but mixtures in which the above configurations predominate are also contemplated.

Reduced Composite Structural Definition of Known MMP Inhibitors

Again without prejudice to the true disclosures of the patent publications listed above, the following reduced composite definition of the disclosed compounds is offered, it being understood that where further information is required concerning the identity of the disclosed compounds, the reader must consult the publication in question. The reduced composite definition is restricted to a core sub-set of the totality of structures encompassed by the patent publications listed above, namely compounds of formula (I) or (Ib):



wherein the five substituents R₁ - R₅ may vary as follows:

- R₁ hydrogen, C₁-C₆ alkyl, C₂-C₆ alkenyl, phenyl, substituted phenyl, phenyl(C₁-C₆ alkyl), heterocyclyl, or a group BSO_nA- wherein n is 0, 1 or 2 and B is hydrogen or a (C₁-C₆) alkyl, phenyl, substituted phenyl, heterocyclyl, C₁-C₆ acyl, phenacyl or substituted phenacyl group, and A represents C₁-C₆ alkyl; amino; protected amino; acylamino; OH; SH; C₁-C₆ alkoxy; C₁-C₆ alkylamino; C₁-C₆ alkylthio; aryl(C₁-C₆ alkyl); amino(C₁-C₆ alkyl); hydroxy(C₁-C₆ alkyl), mercapto(C₁-C₆ alkyl) or carboxy(C₁-C₆ alkyl) wherein the amino-, hydroxy-, mercapto- or carboxyl-group are optionally protected or the carboxyl- group amidated; lower alkyl substituted by maleimido, succinimido, naphthalimido, 2,3-dihydro-1,3-dioxo-1H-benz[d,e]isoquinol-2-yl, carbamoyl, mono(lower alkyl)carbamoyl, di(lower alkyl)carbamoyl, di(lower alkyl)amino, carboxy-lower alkanoylamino, pyrrolidino or morpholino;
- R₂ (C₁-C₆)alkyl; (C₂-C₆)alkenyl; phenyl(C₁-C₆)alkyl; cycloalkyl (C₁-C₆)alkyl; cycloalkenyl (C₁-C₆)alkyl; substituted amino; carboxyl; esterified carboxyl; or a group -Y-X-Z wherein Y is a single bond or (C₁-C₆)alkylene, X is O or S, and Z is (C₁-C₆)alkyl;
- R₃ C₁-C₆ alkyl; benzyl; benzyloxybenzyl; (C₁-C₆ alkoxy)benzyl; benzyloxy(C₁-C₆ alkyl); the characterising group of a natural α amino acid, which may be protected if functional groups are present, eg by acylation of amino groups and amidation of carboxyl groups; a group -[Alk]_nR⁶ where Alk is an C₁-C₆ alkyl or C₂-C₆ alkenyl group optionally interrupted by one or more -O-, or -S-atoms or -N(R⁷)-groups [where R⁷ is a hydrogen atom or a C₁-C₆ alkyl group], n is 0 or 1, and R⁶ is an optionally substituted cycloalkyl or cycloalkenyl group; a benzyl group with one or two substituents in the phenyl ring selected from hydrogen, halogen, cyano amino, amino (C₁-C₆)alkyl, amino di (C₁-C₆)alkyl, amino (C₁-C₆)alkylacyl, aminophenacyl,

amino(substituted)phenacyl, amino acid or derivative thereof, hydroxy, oxy (C_1-C_6)alkyl, oxyacyl, formyl, carboxylic acid, carboxamide, carboxy (C_1-C_6)alkylamide, carboxyphenylamide, carboxy (C_1-C_6)alkyl, hydroxy (C_1-C_6)alkyl, (C_1-C_6)alkyloxy (C_1-C_6)alkyl or acyloxy (C_1-C_6)alkyl, (C_1-C_6)alkylcarboxylic acid, or (C_1-C_6)alkylcarboxy (C_1-C_6)alkyl, amino(C_1-C_6)alkylacyl carboxylic acid or amino(C_1-C_6)alkylcarboxylate; or a benzyl group with one substituent in the phenyl ring selected from groups of formula -OCH₂CN, -OCH₂COR⁸ and -OCH₂CH₂OR⁹, where R⁸ is hydroxyl, (C_1-C_6)oxyalkyl, (C_1-C_6)oxyalkylphenyl, amino, (C_1-C_6)aminoalkyl, (C_1-C_6)aminodialkyl, (C_1-C_6)aminoalkylphenyl, an amino acid or derivative thereof; and R⁹ is (C_1-C_6)alkyl, (C_1-C_6)alkylphenyl, phenyl, substituted phenyl (C_1-C_6)alkylacyl or phenacyl; heterocyclic lower alkyl, either being unsubstituted or mono- or di-substituted with halo, nitro, carboxy, C_1-C_6 alkoxy, cyano, C_1-C_6 alkanoyl, trifluoromethyl C_1-C_6 alkyl, hydroxy, formyl, amino, C_1-C_6 alkyl amino, di- C_1-C_6 alkyl amino, mercapto, C_1-C_6 alkylthio, or mercapto C_1-C_6 alkyl; C_1-C_6 alkyl)phenylmethyl;

R₄ hydrogen, C_1-C_6 alkyl; a group -CHBD wherein B is H and D is H or C_1-C_6 alkyl, C_1-C_6 alkoxy- C_1-C_6 alkyl, di(C_1-C_6 alkoxy)methyl, carboxy, C_1-C_6 alkylcarbonyl, C_1-C_6 alkoxy carbonyl, arylmethoxycarbonyl, C_1-C_6 alkylaminocarbonyl or arylaminocarbonyl; or R₃ and B together form a group (CH₂)_n where n is an integer from 4-11; phenyl(C_1-C_2 alkyl), or trifluoromethylphenyl(C_1-C_2 alkyl); a group -(CH₂)_nA wherein n is an integer from 1 to 6 and A represents the group -NH₂, a substituted acyclic amine, a heterocyclic base, hydroxy, (C_1-C_6)alkoxy, (C_2-C_7)acyloxy, (C_1-C_6)alkylthio, phenylthio, (C_2-C_7)acylamino or an N-pyrrolidone group; an optionally substituted straight or branched alkyl group, optionally interrupted by one or more -O- or -S- atoms or -N(R⁷)-groups [where R⁷ is a hydrogen atom or a C_1-C_6 alkyl group], or aminocarbonyloxy groups; C_1-C_6 alkyl optionally substituted by aryl, amino, protected amino, di(lower alkyl)amino, guanidino, carboxyl, protected carboxyl, carbamoyl, mono(lower alkyl)carbamoyl, di(lower alkyl)carbamoyl, di(lower alkyl)phosphinyl,

dihydroxyphosphinyl, pyrrolidino, piperidino or morpholino;

R₅ hydrogen;

Detailed Description of the Invention

In a first aspect of the invention there is provided the use of a compound forming part of the state of the art by virtue of any of the patent publications listed above in the preparation of an agent for use in the treatment of diseases mediated by TNF.

Without prejudice to the generality of the foregoing, the invention includes the use of a compound according to the general composite structural definition set out above in the preparation of an agent for use in the treatment of diseases mediated by TNF.

More particularly the invention includes the use of a compound according to the general composite structural definition set out above in the preparation of an agent for use in the treatment of diseases mediated by TNF.

In another aspect, this invention concerns a method of management (by which is meant treatment or prophylaxis) of diseases mediated by TNF in mammals, in particular in humans, which method comprises administering to the mammal an effective amount of a compound forming part of the state of the art by virtue of any of the patent publications listed above.

Without prejudice to the generality of the foregoing, the invention includes a method of management (by which is meant treatment or prophylaxis) of diseases mediated by TNF in mammals, in particular in humans, which method comprises administering to the mammal an effective amount of a compound according to the general composite structural definition set out above.

More particularly, the invention includes a method of management (by which is meant treatment or prophylaxis) of diseases mediated by TNF in mammals, in particular in humans, which method comprises administering to the mammal an effective amount of a compound according to the reduced composite structural

definition set out above.

Diseases mediated by TNF include, but are not limited to, inflammation, fever, cardiovascular effects, haemorrhage, coagulation and acute phase response, cachexia and anorexia, acute infections, shock states, graft versus host reactions and autoimmune disease.

Specifically the target for the present invention is inhibition of the release of and the effects of TNF in septic shock, haemodynamic shock or sepsis syndrome, in post ischaemic reperfusion injury, in malaria, mycobacterial infection and meningitis, in psoriasis, in cancer, in cachexia, in fibrotic disease, in congestive heart failure, in graft rejection, in rheumatoid arthritis, in radiation damage and toxicity following administration of immunosuppressive monoclonal antibodies such as OKT3 or CAMPATH-1, in hyperoxic alveolar injury, in autoimmune disease for example AIDS and multiple sclerosis, and in any disease state where TNF is a mediator of host injury.

Specific compounds having TNF inhibitory activity, for use in the present invention are those listed in the Examples below, particularly those listed in Example 2, Group A.

The compounds with which the invention is concerned may be prepared for administration by any route consistent with their physicochemical and pharmacokinetic properties. The compositions thus may be in the form of tablets, capsules, powders, granules, lozenges, liquid or gel preparations, such as oral, topical, or sterile parenteral solutions or suspensions, as appropriate. Tablets and capsules for oral administration may be in unit dose presentation form, and may contain conventional excipients such as binding agents, for example syrup, acacia, gelatin, sorbitol, tragacanth, or polyvinyl-pyrrolidone; fillers for example lactose, sugar, maize-starch, calcium phosphate, sorbitol or glycine; tabletting lubricant, for example magnesium stearate, talc, polyethylene glycol or silica; disintegrants for example potato starch, or acceptable wetting agents such as sodium lauryl sulphate. The tablets may be coated according to methods well known in normal pharmaceutical practice. Oral liquid preparations may be in the

form of, for example, aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, or may be presented as a dry product for reconstitution with water or other suitable vehicle before use. Such liquid preparations may contain conventional additives such as suspending agents, for example sorbitol, syrup, methyl cellulose, glucose syrup, gelatin hydrogenated edible fats; emulsifying agents, for example lecithin, sorbitan monooleate, or acacia; non-aqueous vehicles (which may include edible oils), for example almond oil, fractionated coconut oil, oily esters such as glycerine, propylene glycol, or ethyl alcohol; preservatives, for example methyl or propyl p-hydroxybenzoate or sorbic acid, and if desired conventional flavouring or colouring agents.

The dosage unit for oral administration may contain from about 1 to 250mg, for example from about 25 to 250mg of a compound of general formula I. A suitable daily dose for a mammal may vary widely depending on the condition of the patient. However, a dose of a compound of general formula I of about 0.1 to 300mg/kg body weight, particularly from about 1 to 100mg/kg body weight may be appropriate.

For topical application to the skin, the drug may be made up into a cream, lotion or ointment. Cream or ointment formulations which may be used for the drug are conventional formulations well known in the art, for example as described in standard textbooks of pharmaceutics such as the British Pharmacopoeia.

For topical application to the eye, the drug may be made up into a solution or suspension in a suitable sterile aqueous or non aqueous vehicle. Additives, for instance buffers such as sodium metabisulphite or disodium edetate; preservatives including bactericidal and fungicidal agents such as phenyl mercuric acetate or nitrate, benzalkonium chloride or chlorhexidine, and thickening agents such as hypromellose may also be included.

The dosage for topical administration will of course depend on the size of the area being treated. For the eyes, each dose may typically be in the range from 10 to 100mg of the drug.

The active ingredient may also be administered parenterally in a sterile medium. Depending on the vehicle and concentration used, the drug can either be suspended or dissolved in the vehicle. Advantageously, adjuvants such as a local anaesthetic, preservative and buffering agents can be dissolved in the vehicle.

Although this invention is concerned with the use of certain matrix metalloproteinase inhibitors containing a hydroxamic acid group, known from specific patent publications as described above, it is thought highly probable that those other structural classes of compounds which are known in the art as matrix metalloproteinase inhibitors will, by analogous mechanisms, also have the property of inhibiting the production of TNF, and will therefore also be of use for the treatment of TNF-mediated diseases.

The following examples illustrate the invention, but are not intended to limit the scope in any way. As stated, the known MMP inhibitors made part of the state of the art by virtue of the patent publications listed above have in general the property of inhibiting TNF production, although of course the potency of the compounds *in vitro* and *in vivo* will vary. Compounds which on first testing appear less active, may nonetheless show higher potencies when optimally formulated or when administered at higher dose levels, or when the dosage schedule is optimised.

Example 1 Metalloproteinase inhibitors can prevent the release of TNF from PMA-stimulated human monocytic cell line U937.

The ability of representative compounds, which are inhibitors of metalloproteinases, to inhibit the release of TNF was investigated. The assay is based on the ability of phorbol myristate acetate (PMA) to stimulate the release of TNF α from a human monocytic cell line, U937.

U937 cells cultured in RPMI 1640 medium + 5% foetal calf serum were centrifuged at 1000 x g for 5 minutes and then resuspended in medium at 2 x 10⁶ / ml. 1 ml of cell suspension was aliquoted into individual wells of 24-well plates. Test

compounds were dissolved in dimethyl sulphoxide (DMSO) at a stock concentration of 100mM, then diluted to 50x the final required concentration with RPMI 1640 medium. 20 μ l of the diluted compounds were added to U937 cells in duplicate wells. TNF α release was stimulated by addition of PMA to the cells at a final concentration of 50nM. Appropriate control cultures were set up in duplicate. The plates were incubated for 18 hours at 37°C, 5% CO₂, then centrifuged at 1000 \times g for 5 minutes. A specific ELISA for TNF α obtained from British Bio-technology Products Limited, Abingdon, England was used to measure TNF α levels in the culture supernatants according to the protocol supplied.

The average concentration of test compound which inhibits the release of TNF α by 50% relative to the control culture was assessed as greater than 90 μ M, in the range 10 μ M -90 μ M, or less than 10 μ M.

Group A; Compounds with IC₅₀s less than 10 micromolar.

- 1 3R-(2-(4-methoxyphenyl)-1S-methylcarbamoyl-ethylcarbamoyl)-2S,5-dimethyl-hexanohydroxamic acid.
- 2 2S-(phenylsulfanylmethyl)-3R-(2-phenyl-1S-methylcarbamoyl-ethylcarbamoyl)-5-methyl-hexanohydroxamic acid.
- 3 2S-(thien-2-ylsulfanylmethyl)-3R-(2-phenyl-1S-methylcarbamoyl-ethylcarbamoyl)-5-methyl-hexanohydroxamic acid.
- 4 2S-(benzoylsulfanylmethyl)-3R-(2-phenyl-1S-methylcarbamoyl-ethylcarbamoyl)-5-methyl-hexanohydroxamic acid.
- 5 3R-{2-phenyl-1S-(3-[2-oxo-pyrolidin-1-yl]propylcarbamoyl)-ethylcarbamoyl}-2S,5-dimethyl-hexanohydroxamic acid.
- 6 3R-(2-phenyl-1S-(2-[pyrid-2-yl]ethyl)carbamoyl-ethylcarbamoyl)-2S,5-dimethyl-hexanohydroxamic acid hydrochloride.

- 7 2S-(2,2-dimethylpropionylsulfanylmethyl)-3R-(2-phenyl-1S-methylcarbamoyl-ethylcarbamoyl)-5-methyl-hexanohydroxamic acid.
- 8 3R-(2-phenyl-1S-pyrid-3-ylmethylcarbamoyl-ethylcarbamoyl)-2S,5-dimethyl-hexanohydroxamic acid.
- 9 2S-(phenylsulfonylmethyl)-3R-(2-phenyl-1S-methylcarbamoyl-ethylcarbamoyl)-5-methyl-hexanohydroxamic acid.
- 10 3R-(2-(4-methoxyphenyl)-1S-(2-hydroxyethylcarbamoyl)-ethylcarbamoyl)-2S,5-dimethyl-hexanohydroxamic acid.
- 11 2S-(4-methoxyphenylsulfanylmethyl)-3R-(2-phenyl-1S-methylcarbamoyl-ethylcarbamoyl)-5-methyl-hexanohydroxamic acid.
- 12 sodium 2S-methyl-3R-(2-phenyl-1S-(pyrid-3-ylmethylcarbamoyl)-ethylcarbamoyl)-5-methyl-hexanohydroxamate.
- 13 2S-(3-bromophenylsulfanylmethyl)-3R-(2-phenyl-1S-methylcarbamoyl-ethylcarbamoyl)-5-methyl-hexanohydroxamic acid.
- 14 sodium 2S-methyl-3R-(2-phenyl-1S-(pyrid-4-ylmethylcarbamoyl)-ethylcarbamoyl)-5-methyl-hexanohydroxamate.
- 15 2S-(4-hydroxyphenylsulfanylmethyl)-3R-(2-phenyl-1S-methylcarbamoyl-ethylcarbamoyl)-5-methyl-hexanohydroxamic acid.
- 16 2S-(3-chlorophenylsulfanylmethyl)-3R-(2-phenyl-1S-methylcarbamoyl-ethylcarbamoyl)-5-methyl-hexanohydroxamic acid.
- 17 2S-(phenylsulfonylmethyl)-3R-(2-phenyl-1S-(pyrid-3-ylmethylcarbamoyl)-ethylcarbamoyl)-5-methyl-hexanohydroxamic acid.

- 18 2S-(3-methylphenylsulfanyl methyl)-3R-(2-phenyl-1S-methylcarbamoyl-ethylcarbamoyl)-5-methyl-hexanohydroxamic acid.
- 19 2S-(4-acetamidophenylsulfanyl methyl)-3R-(2-phenyl-1S-methylcarbamoyl-ethylcarbamoyl)-5-methyl-hexanohydroxamic acid.
- 20 2S-(thien-2-ylsulfanyl methyl)-3R-(2-(4-methoxyphenyl)-1S-methylcarbamoyl-ethylcarbamoyl)-5-methyl-hexanohydroxamic acid.
- 21 2S-(4-[3-ethoxycarbonyl-propionylamino]phenylsulfanyl methyl)-3R-(2-phenyl-1S-methylcarbamoyl-ethylcarbamoyl)-5-methyl-hexanohydroxamic acid.
- 22 2S-(methoxycarbonylmethylsulfanyl methyl)-3R-(2-phenyl-1S-methylcarbamoyl-ethylcarbamoyl)-5-methyl-hexanohydroxamic acid.
- 23 3R-(2-(4-acetamidophenyl)-1S-methylcarbamoyl-ethylcarbamoyl)-5-methyl-hexanohydroxamic acid.
- 24 3R-(2-(4-dimethylcarbamoylmethoxyphenyl)-1S-methylcarbamoyl-ethylcarbamoyl)-5-methyl-hexanohydroxamic acid.
- 25 2S-(thien-2-ylsulfanyl methyl)-3R-(3-methoxycarbonyl-1S-methylcarbamoyl-propylcarbamoyl)-5-methyl-hexanohydroxamic acid.
- 26 dilithio-2S-methyl-3R-(2-(4-carboxymethylcarbamoylmethoxyphenyl)-1S-methylcarbamoyl-ethylcarbamoyl)-5-methyl-hexanohydroxamate.
- 27 dilithio-2S-(thien-2-ylsulphanyl methyl)-3R-(2-(4-carboxymethylcarbamoylmethoxyphenyl)-1S-methylcarbamoyl-ethylcarbamoyl)-5-methyl-hexanohydroxamate.
- 28 2S-(thien-2-ylsulphanyl methyl)-3R-(2-(4-methoxycarbonylmethoxyphenyl)-1S-methylcarbamoyl-ethylcarbamoyl)-5-methyl-hexanohydroxamic acid.

- 29 2S-(thien-2-ylsulphanyl methyl)-3R-(2-(4-carboxymethoxyphenyl)-1S-methylcarbamoyl-ethylcarbamoyl)-5-methyl-hexanohydroxamic acid.
- 30 2S-(thien-2-ylsulphanyl methyl)-3R-(5-(1,1-dimethyl-ethoxycarbamoyl)-1S-methylcarbamoyl-pentylcarbamoyl)-5-methyl-hexanohydroxamic acid.
- 31 2S-(thien-2-ylsulphanyl methyl)-3R-(2-phenyl-1S-(pyrid-3-ylmethylcarbamoyl)-ethylcarbamoyl)-5-methyl-hexanohydroxamic acid.
- 32 2S-(4-hydroxyphenylsulphanyl methyl)-3R-(5-(1,1-dimethyl-ethoxycarbamoyl)-1S-methylcarbamoyl-pentylcarbamoyl)-5-methyl-hexanohydroxamic acid.
- 33 3R-(5-(1,1-dimethyl-ethoxycarbamoyl)-1S-methylcarbamoyl-pentylcarbamoyl)-2S,5-dimethyl-hexanohydroxamic acid.
- 34 2S-(4-hydroxyphenylsulphanyl methyl)-3R-(2-phenyl-1S-(pyrid-3-ylmethylcarbamoyl)-ethylcarbamoyl)-5-methyl-hexanohydroxamic acid.
- 35 2S-(4-aminophenylsulphanyl methyl)-3R-(2-phenyl-1S-(pyrid-3-ylmethylcarbamoyl)-ethylcarbamoyl)-5-methyl-hexanohydroxamic acid.
- 36 2S-(methoxycarbonylmethylsulphanyl methyl)-3R-(2-phenyl-1S-(pyrid-3-ylmethylcarbamoyl)-ethylcarbamoyl)-5-methyl-hexanohydroxamic acid.
- 37 Dilithio-2S-(thien-2-ylsulphanyl methyl)-3R-(2-(4-[3-carboxypropionyl]amino-phenyl)-1S-methylcarbamoyl-ethylcarbamoyl)-5-methyl-hexanohydroxamate.
- 38 Dilithio-2S-(4-aminophenylsulphanyl methyl)-3R-(2-[4-(3-carboxypropionyl)amino-phenyl]-1S-methylcarbamoyl-ethylcarbamoyl)-5-methyl-hexanohydroxamate.
- 39 Dilithio-2S-(4-hydroxyphenylsulphanyl methyl)-3R-(2-[4-(3-carboxypropionyl)amino-phenyl]-1S-methylcarbamoyl-ethylcarbamoyl)-5-methyl-

hexanohydroxamate.

40 2S-hydroxy-3R-(2-phenyl-1S-methylcarbamoyl-ethylcarbamoyl)-5-methyl-hexanohydroxamic acid.

41 2R-(3-bromophenylmethyl)-3R-(2-phenyl-1S-methylcarbamoyl-ethylcarbamoyl)-5-methyl-hexanohydroxamic acid.

42 2R-(3-bromophenylmethyl)-3R-(3-methoxycarbonyl-1S-methylcarbamoyl-propylcarbamoyl)-5-methyl-hexanohydroxamic acid.

43 2S-(4-hydroxyphenylsulphanylmethyl)-3R-(5-[1,1-dimethyl-ethoxycarbonyl],[4-hydroxyphenylsulfanylmethyl]-amino-1S-methylcarbamoyl-pentylcarbamoyl)-5-methyl-hexanohydroxamic acid.

44 2S-(thien-2-ylsulfanylmethyl)-3R-(2-naph-2-yl-1S-methylcarbamoyl-ethylcarbamoyl)-5-methyl-hexanohydroxamic acid.

45 2S-(3,4-dimethoxyphenylsulfanylmethyl)-3R-(2-phenyl-1S-methylcarbamoyl-ethylcarbamoyl)-5-methyl-hexanohydroxamic acid.

46 2S-(4-[thien-2-yl]acetamidophenylsulfanylmethyl)-3R-(2-phenyl-1S-methylcarbamoyl-ethylcarbamoyl)-5-methyl-hexanohydroxamic acid.

47 2S-hydroxy-3R-(2-oxo-aza-tridecan-3-ylcarbamoyl)-5-methyl-hexanohydroxamic acid.

48 2S-(thien-2-ylsulfanylmethyl)-3R-(2-phenyl-1S-[2,2-dimethylpropionyloxymethyl]-methyl-carbamoyl-ethylcarbamoyl)-5-methyl-hexanohydroxamic acid.

49 2S-(4-hydroxyphenylsulfanylmethyl)-3R-(2R-hydroxy-1S-methylcarbamoyl-propylcarbamoyl)-5-methyl-hexanohydroxamic acid.

- 50 2S-(4-hydroxyphenylsulfanylmethyl)-3R-(5-acetamido-1S-methylcarbamoyl-pentylcarbamoyl)-5-methyl-hexanohydroxamic acid.
- 51 2S-(ethylsulfanylmethyl)-3R-(2-cyclohexyl-1S-methylcarbamoyl-ethylcarbamoyl)-5-methyl-hexanohydroxamic acid.
- 52 2S-(4-hydroxyphenylsulfanylmethyl)-3R-(2-[1,1-dimethylethoxycarbonyl]-1S-methylcarbamoyl-ethylcarbamoyl)-5-methyl-hexanohydroxamic acid.
- 53 2S-(4-hydroxyphenylsulfanylmethyl)-3R-(3-methoxycarbonyl-1S-methylcarbamoyl-propylcarbamoyl)-5-methyl-hexanohydroxamic acid.
- 54 2S-(4-hydroxyphenylsulfanylmethyl)-3R-(3-[1,1-dimethylethoxycarbonyl]-1S-methylcarbamoyl-propylcarbamoyl)-5-methyl-hexanohydroxamic acid.
- 55 2S-(ethylsulfanylmethyl)-3R-(3-methoxycarbonyl-1S-methylcarbamoyl-propylcarbamoyl)-5-methyl-hexanohydroxamic acid.
- 56 2S-(4-aminophenylsulfanylmethyl)-3R-(3-methoxycarbonyl-1S-methylcarbamoyl-propylcarbamoyl)-5-methyl-hexanohydroxamic acid.
- 57 2S-(4-methoxyphenylsulfanylmethyl)-3R-(3-methoxycarbonyl-1S-methylcarbamoyl-propylcarbamoyl)-5-methyl-hexanohydroxamic acid.
- 58 2S-(benzylsulfanylmethyl)-3R-(3-methoxycarbonyl-1S-methylcarbamoyl-propylcarbamoyl)-5-methyl-hexanohydroxamic acid.
- 59 2S-(benzyloxy)-3R-(2-phenyl-1S-methylcarbamoyl-ethylcarbamoyl)-5-methyl-hexanohydroxamic acid.
- 60 2S-(4-hydroxyphenylsulfanylmethyl)-3R-(3-carboxy-1S-methylcarbamoyl-propylcarbamoyl)-5-methyl-hexanohydroxamic acid.
- 61 2S-(1,1-dimethylethylsulfanylmethyl)-3R-(3-methoxycarbonyl-1S-

methylcarbamoyl-propylcarbamoyl)-5-methyl-hexanohydroxamic acid.

62 2S-(prop-2-enyloxy)-3R-(2-phenyl-1S-methylcarbamoyl-ethylcarbamoyl)-5-methyl-hexanohydroxamic acid.

63 2S-hydroxy-3R-(3-methoxycarbonyl-1S-methylcarbamoyl-propylcarbamoyl)-5-methyl-hexanohydroxamic acid.

64 2S-(prop-2-enyloxy)-3R-(2-cyclohexyl-1S-methylcarbamoyl-ethylcarbamoyl)-5-methyl-hexanohydroxamic acid.

65 2S-(4-hydroxyphenylsulfanylmethyl)-3R-(3-methylcarbamoyl-1S-methylcarbamoyl-propylcarbamoyl)-5-methyl-hexanohydroxamic acid.

66 2S-(4-hydroxyphenylsulfanylmethyl)-3R-(2-phenyl-1S-methylcarbamoyl-ethylcarbamoyl)-4-methyl-pentanohydroxamic acid.

67 3R-(2-phenyl-1S-[1-hydroxy-2,2-dimethyl-prop-3-yl]carbamoyl-ethylcarbamoyl)-5-methyl-hexanohydroxamic acid.

68 2-(1S-formylhydroxyamino-butyl)-4-methyl-pentanoic acid-[1-(1S-methylcarbamoyl)-2-{4-methoxyphenyl}-ethyl]-amide

Group B: Compounds with IC₅₀s between 10 and 90 micromolar.

69 3S-(2(4-methoxyphenyl)-1S-methylcarbamoyl-ethylcarbamoyl)-2S,5-dimethyl-hexanohydroxamic acid.

70 3R-(2(4-methoxyphenyl)-1S-methylcarbamoyl-ethylcarbamoyl)-5-methyl-hexanohydroxamic acid.

71 3R-(2-phenyl-1S-carboxy-ethylcarbamoyl)-5-methyl-hexanohydroxamic acid.

72 3R-(2-phenyl-1S-[3-imidazo-1-yl-propylcarbamoyl]-ethylcarbamoyl)-5-

methyl-hexanohydroxamic acid.

73 3R-(2-phenyl-1S-[2-(1-methylpyrrol-2-yl)ethylcarbamoyl]-ethylcarbamoyl)-5-methyl-hexanohydroxamic acid.

74 3R-(2-phenyl-1S-[2-oxo-pyrolid-1-yl]propylcarbamoyl-ethylcarbamoyl)-5-methyl-hexanohydroxamic acid.

75 2S-(2,4-dimethylphenylsulfanyl methyl)-3R-(2-phenyl-1S-methylcarbamoyl-ethylcarbamoyl)-5-methyl-hexanohydroxamic acid.

76 3R-(2-phenyl-1S-methylcarbamoyl-ethylcarbamoyl)-5-methyl-hexanohydroxamic acid.

77 3R-(2-[4-cyanophenyl]-1S-methylcarbamoyl-ethylcarbamoyl)-5-methyl-hexanohydroxamic acid.

78 3R-(2-[4-methoxycarbonylmethylcarbamoylmethoxyphenyl]-1S-methylcarbamoyl-ethylcarbamoyl)-5-methyl-hexanohydroxamic acid.

79 3R-(2-[4-carboxymethylcarbamoylmethoxyphenyl]-1S-methylcarbamoyl-ethylcarbamoyl)-5-methyl-hexanohydroxamic acid.

80 3R-(2-[4-carbamoylmethoxyphenyl]-1S-methylcarbamoyl-ethylcarbamoyl)-5-methyl-hexanohydroxamic acid.

81 3R-(2-[4-aminophenyl]-1S-methylcarbamoyl-ethylcarbamoyl)-5-methyl-hexanohydroxamic acid.

82 3R-(2-phenyl-1S-[2-phenyl-1R-methyl-ethylcarbamoyl]-ethylcarbamoyl)-5-methyl-hexanohydroxamic acid.

83 dilithio-3R-(2-[4-{3-carboxypropionyl}amino-phenyl]-1S-methylcarbamoyl-ethylcarbamoyl)-2S,5-dimethyl-hexanohydroxamate.

84 2R-(4-hydroxyphenylsulfanylmethyl)-3R-(3-methoxycarbonyl-1S-methylcarbamoyl-propylcarbamoyl)-5-methyl-hexanohydroxamic acid.

85 3R-(2-phenyl-1S-[2-amino-ethylcarbamoyl]-ethylcarbamoyl)-5-methyl-hexanohydroxamic acid.

86 3R-(2-phenyl-1S-[3-{morpholin-4-yl}-propylcarbamoyl]-ethylcarbamoyl)-4-cyclopentyl-butanohydroxamic acid.

87 3R-(2-phenyl-1S-methylcarbamoyl-ethylcarbamoyl)-5-phenyl-pentanohydroxamic acid.

88 3R-(2,2-dimethyl-1S-methylcarbamoyl-propylcarbamoyl)-5-methyl-hexanohydroxamic acid.

89 2S-thiomethyl-3R-(3-methoxycarbonyl-1S-methylcarbamoyl-propylcarbamoyl)-5-methyl-hexanohydroxamic acid.

90 3R-(6-carbamoyl-1S-methylcarbamoyl-hexylcarbamoyl)-5-methyl-hexanohydroxamic acid.

91 3R-(4-carbamoyl-1S-methylcarbamoyl-butylcarbamoyl)-5-methyl-hexanohydroxamic acid.

92 2S-(4-phthalimido-butyl)-3S-(3-methyl-1S-ethoxycarbonylmethylcarbamoyl-butylcarbamoyl)-5-methyl-hexanohydroxamic acid.

93 2S-(4-phthalimido-butyl)-3R-(3-methyl-1S-ethoxycarbonylmethylcarbamoyl-butylcarbamoyl)-5-methyl-hexanohydroxamic acid.

94 3R-(2-phenyl-1S-phenylcarbamoyl-ethylcarbamoyl)-5-methyl-hexanohydroxamic acid.

95 2-formylhydroxyaminomethyl-4-methyl-pentanoic acid-[1-(1S-methylcarbamoyl)-2-{4-methoxyphenyl}-ethyl]-amide

96 2-(1S-formylhydroxyamino-ethyl)-4-methyl-pentanoic acid-[1-(1S-methylcarbamoyl)-2-{4-carboxymethoxyphenyl}-ethyl]-amide

97 2-(1S-formylhydroxyamino-ethyl)-4-methyl-pentanoic acid-[1-(1S-methylcarbamoyl)-2-{4-aminophenyl}-ethyl]-amide

98 3R-(2-phenyl-1S-[pyrid-3-ylmethylcarbamoyl]-ethylcarbamoyl)-5-methyl-hexanohydroxamic acid.

99 2S-(thien-2-ylsulfonylmethyl)-3R-(2-phenyl-1S-methylcarbamoyl-ethylcarbamoyl)-5-methyl-hexanohydroxamic acid.

Group C: Compounds with IC₅₀s greater than 90 micromolar.

100 3R-(2-(4-methoxyphenyl)-1S-dimethylcarbamoyl-ethylcarbamoyl)-5-methyl-hexanohydroxamic acid.

101 3R-(2-oxo-1,2,3,4-tetrahydro-quinoli-3-ylcarbamoyl)-5-methyl-hexanohydroxamic acid.

102 3R-(5-amino-1S-methylcarbamoyl-pentylcarbamoyl)-5-methyl-hexanohydroxamic acid.

103 3R-(3-carboxy-1S-[1S-phenyl-ethyl]carbamoyl-propylcarbamoyl)-5-methyl-hexanohydroxamic acid.

104 3R-(2-phenyl-1S-[2-carboxyethylcarbamoyl]-ethylcarbamoyl)-5-methyl-hexanohydroxamic acid.

105 3R-(2-phenyl-1S-[2-phenyl-1S-methoxycarbonyl-ethylcarbamoyl]-ethylcarbamoyl)-5-methyl-hexanohydroxamic acid.

106 3R-(2-pyrazol-1-yl-1S-methylcarbamoyl-ethylcarbamoyl)-5-methyl-hexanohydroxamic acid.

107 3R-(2-phenyl-1R-carboxy-ethylcarbamoyl)-5-methyl-hexanohydroxamic acid.

108 3R-(3-methyl-1S-[1R-ethoxycarbonyl-ethylcarbamoyl]-butylcarbamoyl)-5-methyl-hexanohydroxamic acid.

109 2S-propyl-3R-(2-phenyl-1S-methylcarbamoyl-ethylcarbamoyl)-5-methyl-hexanohydroxamic acid.

110 3R-(2-phenyl-1S-[1,1-dimethylethylcarbamoyl]-ethylcarbamoyl)-5-methyl-hexanohydroxamic acid.

111 3R-(2-methyl-1S-methylcarbamoyl-propylcarbamoyl)-5-methyl-hexanohydroxamic acid.

112 3R-(2-phenyl-1S-[1,1-dimethyl-propylcarbamoyl]-ethylcarbamoyl)-5-methyl-hexanohydroxamic acid.

113 3R-(2-phenyl-1S-dimethylcarbamoyl-ethylcarbamoyl)-4-methyl-pentanohydroxamic acid.

114 3R-(2-phenyl-1S-[pyrid-3-ylmethylcarbamoyl]-ethylcarbamoyl)-5-methyl-hexanohydroxamic acid.

Example 2 Metalloproteinase inhibitors can prevent the release of TNF from LPS-stimulated whole blood cultures.

The ability of representative compounds, which are inhibitors of metalloproteinases, to inhibit the release of TNF was investigated. A modified whole blood culture system was used (Wilson BMG, Severn A, Rapson NT, Chana J & Hopkins P. (1991) J. Immunol. Meths. 139:233-240). The compounds tested

were nos 1, 69, 24, 78, 2, and 34.

Blood was removed from healthy volunteers by venepuncture into heparinised containers, diluted to 1:4 with RPMI 1640 medium and aliquoted into 1.5ml culture wells. Test compounds were added directly to each well. Control cultures containing RPMI-1640 alone, LPS at 100ng/ml and LPS plus diluent were included in each experiment. Test compounds at dilutions from 100 μ M final concentration were added to culture wells containing 100ng/ml LPS. After 24hrs incubation at 37°C, supernatants were removed, centrifuged at 1200rpm for 10 mins and assayed for TNF α . A specific ELISA for TNF α (British Bio-technology Products) was used to measure TNF levels. The specificity of inhibition was confirmed by measuring the release of IL-1b and IL-6 from the same cultures using specific ELISAs (British Bio-technology Products Limited, Abingdon, England). The production of these latter cytokines was not inhibited by the metalloproteinase inhibitors.

The test compounds specifically inhibit the release of TNF from LPS-stimulated blood cells into the supernatant at concentrations below 100 μ M.

Example 3

Test compounds were assessed for their ability to inhibit release of endotoxin induced TNF production *in vivo*.

Male CD rats (Charles River, UK) weighing between 300g - 400g were anaesthetised with an intra-peritoneal injection of a mixture of 62.5mg.kg $^{-1}$ thiopental and 22.5mg.kg $^{-1}$ sodium pentobarbitone (Thiopental, Sigma Chemical Co., UK; Sodium pentobarbitone (Sagatal), May and Baker., UK). The trachea was exposed and cannulated to allow spontaneous respiration through a patent airway. The left jugular vein was exposed and cannulated for the administration of LPS. The right femoral artery was exposed and cannulated for withdrawal of blood samples.

Blood samples (1ml) were removed 5 minutes prior to bolus administration of either LPS (*E. coli* serotype 0111:b4, Difco Laboratories, USA) or saline (NaCL 0.9% w/v) and at intervals of 1 hour and 2 hours after LPS. Removed blood was replaced with equal amounts of saline.

Rats were administered LPS at a dose of 500 μ g.kg⁻¹ which resulted in a marked increase in TNF α levels in comparison to saline controls. The peak of TNF release was at the 1 hour timepoint. TNF levels were expressed in units/ml where 1 unit is the equivalent of 1pg of mouse TNF α .

Rat serum TNF levels were measured using a mouse TNF α reference standard. The commercial ELISA kits were from Genzyme.

Test compounds were administered intraperitoneally to anaesthetised rats at a dose of 10mg.kg⁻¹, 1 hour prior to LPS administration.

The results are expressed as the % inhibition of maximal TNF elevation relative to control:

Compound Tested	% Inhibition of Maximal TNF Elevation
87	4
99	55
23	35
93	43
1	0
74	28
70	37
98	28
88	66

In a variation of the above methodology, the infusion of compound 29, 1mg/kg/hr, 15 minutes prior to the administration of LPS, 500 μ g.kg⁻¹, for 2 hours resulted in a 60% inhibition of maximal TNF elevation relative to control.

Claims:

1. The use of a compound forming part of the state of the art by virtue of any of the following patent publications:

US 4599361 ("Searle")

EP-A-0236872 ("Roche 1")

EP-A-0274453 ("Bellon")

WO 90/05716 (British Bio-technology) ("BBL1")

WO 90/05719 (British Bio-technology) ("BBI 2")

WO 91/02716 (British Bio-technology) ("BBI 3")

EP-A-0489577 ("Celltech 1")

EP-A-0489579 ("Celltech 2")

EP-A-0497192 ("Roche 2")

WO 92/13831 (British Bio-technology) ("BBI 4")

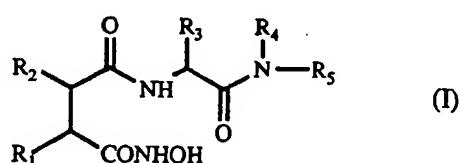
WO 92/22523 (Research Corporation Technologies) ("RCT")

WQ 93/09090 ("Yamaneuchi")

WO 93/09097 ("Sankyo")

in the preparation of an agent for use in the treatment of diseases mediated by TNF

2. The use as claimed in claim 1 of a compound forming part of the state of the art by virtue of the patent publication US 4599361 ("Searle"), namely a compound of formula (I)



wherein the five substituents R₁ - R₅ may vary as follows:

R₁ hydrogen, C₁-C₆ alkyl, phenyl, phenyl(C₁-C₆ alkyl):

R₂ hydrogen, phenyl, phenyl(C₁-C₆ alkyl), cycloalkyl, cycloalkyl(C₁-C₆ alkyl):

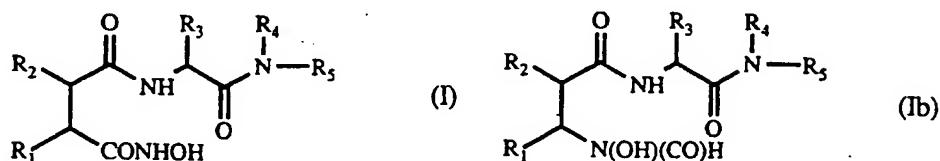
R₃: C₁-C₆ alkyl, benzyl, benzyloxybenzyl, (C₁-C₆ alkoxy)benzyl

benzyloxy(C₁-C₆ alkyl);

R₄ C₁-C₆ alkyl;

R₅ hydrogen;

3. The use as claimed in claim 1 of a compound forming part of the state of the art by virtue of the patent publication EP-A-0236872 ("Roche 1"), namely a compound of formula (I) or (Ib)



wherein the five substituents R_1 - R_5 may vary as follows:

R₁ hydrogen, NH₂, OH, SH, C₁-C₆ alkyl, C₁-C₆ alkoxy, C₁-C₆ alkylamino, C₁-C₆ alkylthio or aryl(C₁-C₆ alkyl) group or amino-C₁-C₆ alkyl, hydroxy-C₁-C₆ alkyl, mercapto-C₁-C₆ alkyl or carboxy-C₁-C₆ alkyl where the amino-, hydroxy-, mercapto or carboxyl group can be protected, the amino group acylated or the carboxyl group amidated;

R₂ C₂-C₅ alkyl;

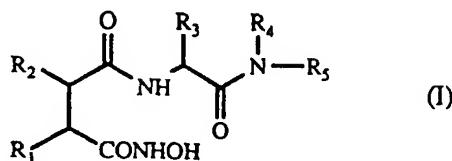
R_3 the characterising group of a natural α amino acid, which may be protected if functional groups are present, eg acylation of amino groups and amidation of carboxylgroups, with the proviso that R_3 is not H or methyl:

R₄ a group -CHBD wherein B is H or methyl and D is H or C₁-C₆ alkyl, C₁-C₆ alkoxy-C₁-C₆ alkyl, di(C₁-C₆ alkoxy)methyl, carboxy, C₁-C₆alkylcarbonyl, C₁-C₆ alcoxycarbonyl, arylmethoxycarbonyl, C₁-C₆ alkylaminocarbonyl or arylaminocarbonyl, or B and D together form a trimethylene group; or R₃ and B together form a group (CH₂)_n where n is an integer from 4-11;

R_5 H or methyl;

4. The use as claimed in claim 1 of a compound forming part of the state of

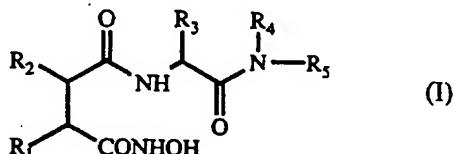
the art by virtue of the patent publication EP-A-0274453 ("Bellon"), namely a compound of formula (I)



wherein the five substituents R₁ - R₅ may vary as follows:

- R₁ hydrogen;
- R₂ isobutyl;
- R₃ isopropyl, 4-aminobutyl, n-butyl, 2-methylmercaptoethyl;
- R₄ hydrogen, phenyl(C₁-C₂ alkyl), or trifluoromethylphenyl(C₁-C₂ alkyl);
- R₅ hydrogen;

5. The use as claimed in claim 1 of a compound forming part of the state of the art by virtue of the patent publication WO 90/05716 (British Bio-technology) ("BBL1"), namely a compound of formula (I)



wherein the five substituents R₁ - R₅ may vary as follows:

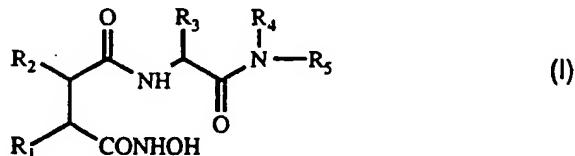
- R₁ hydrogen, C₁-C₆ alkyl, C₂-C₆ alkenyl, phenyl, phenyl(C₁-C₆ alkyl), or a group BSO_nCH₂- wherein n is 0, 1 or 2 and B is hydrogen or a (C₁-C₆) alkyl, phenyl, substituted phenyl, phenyl(C₁-C₆)alkyl, or heterocyclil group;
- R₂ hydrogen atom or an (C₁-C₆)alkyl, (C₂-C₆)alkenyl, phenyl(C₁-C₆)alkyl, cycloalkyl (C₁-C₆)alkyl or cycloalkenyl (C₁-C₆)alkyl group;
- R₃ an amino acid residue with R or S stereochemistry or a (C₁-C₆)alkyl, benzyl, (C₁-C₆)alkoxy benzyl or benzyloxy (C₁-C₆)alkyl group;
- R₄ a group -(CH₂)_nA wherein n is an integer from 1 to 6 and A represents

the group

-NH₂, a substituted acyclic amine or a heterocyclic base;

R₅ a hydrogen atom or a methyl group;

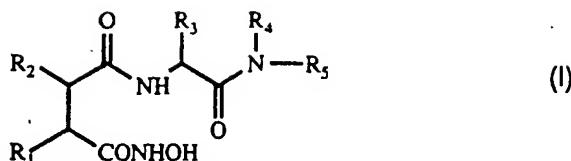
6. The use as claimed in claim 1 of a compound forming part of the state of the art by virtue of the patent publication WO 90/05719 (British Bio-technology) ("BBL2"), namely a compound of formula (I)



wherein the five substituents R₁ - R₅ may vary as follows:

- R₁ a group BSO_nA- wherein n is 0, 1 or 2 and B is hydrogen or an alkyl, phenyl, substituted phenyl, phenylalkyl, heterocyclyl, alkylcarbonyl, phenacyl or substituted phenacyl group, and A represents a hydrocarbon chain optionally substituted with one or more alkyl, phenyl, or substituted phenyl groups;
- R₂ a hydrogen atom or an alkyl, alkenyl, phenylalkyl, cycloalkylalkyl or cycloalkenylalkyl group;
- R₃ an amino acid residue with R or S stereochemistry or a (C₁-C₆)alkyl, benzyl, (C₁-C₆ alkoxy) benzyl or benzyloxy (C₁-C₆ alkyl) group;
- R₄ a hydrogen atom or an alkyl group;
- R₅ a hydrogen atom or a methyl group;

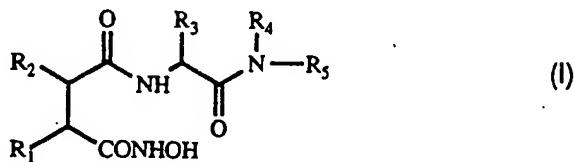
7. The use as claimed in claim 1 of a compound forming part of the state of the art by virtue of the patent publication WO 91/02716 (British Bio-technology) ("BBL3"), namely a compound of formula (I)



wherein the five substituents R_1 - R_5 may vary as follows:

- | | |
|-------|--|
| R_1 | hydrogen or a (C_1 - C_6) alkyl, (C_2 - C_6) alkenyl, phenyl, phenyl(C_1 - C_6)alkyl, (C_1 - C_6)alkylthiomethyl, phenylthiomethyl, substituted phenylthiomethyl, phenyl(C_1 - C_6)alkylthiomethyl or heterocyclithiomethyl group; |
| R_2 | a hydrogen atom or an (C_1 - C_6)alkyl, (C_2 - C_6)alkenyl, phenyl(C_1 - C_6)alkyl, cycloalkyl
(C_1 - C_6)alkyl or cycloalkenyl (C_1 - C_6)alkyl group; |
| R_3 | an amino acid residue with R or S stereochemistry or a (C_1 - C_6)alkyl, benzyl, (C_1 - C_6)alkoxy benzyl, benzyloxy (C_1 - C_6)alkyl or benzyloxybenzyl group; |
| R_4 | a group $(CH_2)_nA$ wherein n is an integer from 1 to 6 and A represents a hydroxy, (C_1 - C_6)alkoxy, (C_2 - C_7)acyloxy, (C_1 - C_6)alkylthio, phenylthio, (C_2 - C_7)acylamino or N-pyrrolidone group; |
| R_5 | a hydrogen atom or a methyl group; |

8. The use as claimed in claim 1 of a compound forming part of the state of the art by virtue of the patent publication EP-A-0489577 ("Celltech 1"), namely a compound of formula (I)



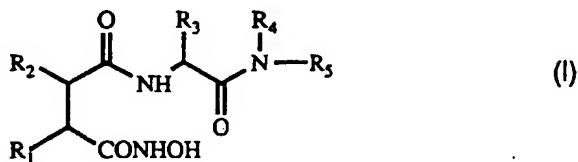
wherein the five substituents R_1 - R_5 may vary as follows:

- R₁ . optionally substituted alkyl, alkenyl, aryl, aralkyl, heteroaralkyl, or

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- heteroarylthioalkyl;
- R₂ optionally substituted alkyl, alkenyl, cycloalkyl, aryl, aralkyl, aralkoxy, or aralkylthio group, or an amino, substituted amino, carboxyl, or esterified carboxyl group;
- R₃ (a group -[Alk]_nR⁶ where Alk is an alkyl or alkenyl group optionally interrupted by one or more -O-, or -S- atoms or -N(R⁷)-groups [where R⁷ is a hydrogen atom or a C₁-C₆ alkyl group], n is zero or an integer 1, and R⁶ is an optionally substituted cycloalkyl or cycloalkenyl group;
- R₄ a hydrogen atom, an optionally substituted straight or branched alkyl group, optionally interrupted by one or more -O- or -S- atoms or -N(R⁷)-groups [where R⁷ is a hydrogen atom or a C₁-C₆ alkyl group], or aminocarbonyloxy groups; or R⁴ and R⁵ together with the nitrogen atom to which they are attached may form an optionally substituted C₃-C₆ cyclic amino group optionally possessing one or more other heteroatoms selected from -O- or -S- or -N(R⁷)-groups [where R⁷ is a hydrogen atom or a C₁-C₆ alkyl group];
- R₅ a hydrogen atom, an optionally substituted straight or branched alkyl group, optionally interrupted by one or more -O- or -S- atoms or -N(R⁷)-groups [where R⁷ is a hydrogen atom or a C₁-C₆ alkyl group], or aminocarbonyloxy groups;

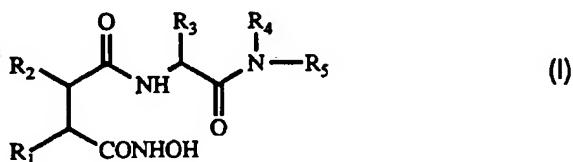
9. The use as claimed in claim 1 of a compound forming part of the state of the art by virtue of the patent publication EP-A-0489579 ("Celltech 2"), namely a compound of formula (I)



wherein the five substituents R₁ - R₅ may vary as follows:

- R₁ optionally substituted alkyl, alkenyl, aryl, aralkyl, heteroaralkyl, or heteroarylthioalkyl;
- R₂ optionally substituted phenylethyl, phenylpropyl or phenylbutyl group;
- R₃ optionally substituted alkyl or alkenyl group optionally interrupted by one or more -O-, or -S- atoms or -N(R⁷)-groups [where R⁷ is a hydrogen atom or a C₁-C₆ alkyl group];
- R₄ a hydrogen atom, an optionally substituted straight or branched alkyl group, optionally interrupted by one or more -O- or -S- atoms or -N(R⁷)-groups [where R⁷ is a hydrogen atom or a C₁-C₆ alkyl group], or aminocarbonyloxy groups; or R⁴ and R⁵ together with the nitrogen atom to which they are attached may form an optionally substituted C₃-C₆ cyclic amino group optionally possessing one or more other heteroatoms selected from -O- or -S- or -N(R⁷)-groups [where R⁷ is a hydrogen atom or a C₁-C₆ alkyl group];
- R₅ a hydrogen atom, an optionally substituted straight or branched alkyl group, optionally interrupted by one or more -O- or -S- atoms or -N(R⁷)-groups [where R⁷ is a hydrogen atom or a C₁-C₆ alkyl group], or aminocarbonyloxy groups;

10. The use as claimed in claim 1 of a compound forming part of the state of the art by virtue of the patent publication EP-A-0497192 ("Roche 2"), namely a compound of formula (I)



wherein the five substituents R₁ - R₅ may vary as follows:

- R₁ hydrogen, amino, protected amino, acylamino, or lower alkyl optionally substituted

by aryl, hydroxy, protected hydroxy, amino, protected amino, acylamino, maleimido, succinimido, naphthalimido, 2,3-dihydro-1,3-dioxo-1H-benz[d,e]isoquinol-2-yl, carboxy, protected carboxy, carbamoyl, mono(lower alkyl)carbamoyl, di(lower alkyl)carbamoyl, di(lower alkyl)amino, carboxy-lower alkanoylamino, pyrrolidino or morpholino;

R₂ isobutyl;

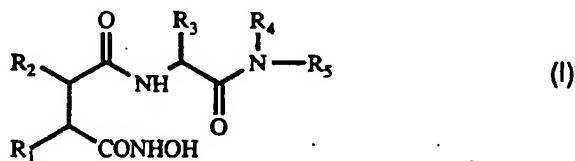
R₃ tert-butyl;

R₄ hydrogen or lower alkyl optionally substituted by aryl, amino, protected amino,

di(lower alkyl)amino, guanidino, carboxyl, protected carboxyl, carbamoyl, mono(lower alkyl)carbamoyl, di(lower alkyl)carbamoyl, di(lower alkyl)phosphinyl, dihydroxyphosphinyl, pyrrolidino, piperidino or morpholino;

R₅ hydrogen;

11. The use as claimed in claim 1 of a compound forming part of the state of the art by virtue of the patent publication WO 92/13831 (British Bio-technology) ("BBL4"), namely a compound of formula (I)



wherein the five substituents R₁ - R₅ may vary as follows:

R₁ hydrogen (C₁-C₆) alkyl, phenyl, substituted phenyl, phenyl(C₁-C₆)alkyl, or heterocyclil; or R₁ represents ASO_nR₇ wherein A represents a (C₁-C₆) hydrocarbon chain, optionally substituted with one or more (C₁-C₆)alkyl, phenyl or substituted phenyl groups and n=0, 1 or 2; R₇ is (C₁-C₆)alkyl, phenyl, substituted phenyl, phenyl (C₁-C₆) alkyl, heterocyclil, (C₁-C₆)alkyl, (C₁-C₆)acyl, thieryl or phenacyl;

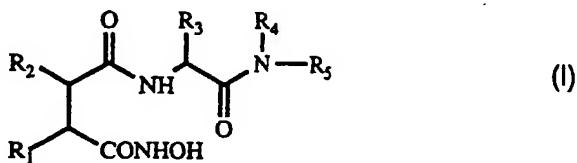
R₂ hydrogen or an (C₁-C₆)alkyl, (C₂-C₆)alkenyl, phenyl(C₁-C₆)alkyl, or

cycloalkyl(C₁

-C₆)alkyl group;

- R₃ a benzyl group with one or two substituents in the phenyl ring selected from hydrogen, halogen, cyano amino, amino (C₁-C₆)alkyl, amino di (C₁-C₆)alkyl, amino (C₁-C₆)alkylacyl, aminophenacyl, amino(substituted)phenacyl, amino acid or derivative thereof, hydroxy, oxy (C₁-C₆)alkyl, oxyacyl, formyl, carboxylic acid, carboxamide, carboxy (C₁-C₆)alkylamide, carboxyphenylamide, carboxy (C₁-C₆)alkyl, hydroxy (C₁-C₆)alkyl, (C₁-C₆)alkyloxy (C₁-C₆)alkyl or acyloxy (C₁-C₆)alkyl, (C₁-C₆)alkylcarboxylic acid, or (C₁-C₆)alkylcarboxy (C₁-C₆)alkyl, amino(C₁-C₆)alkylacyl carboxylic acid or amino(C₁-C₆)alkylcarboxylate; or a benzyl group with one substituent in the phenyl ring selected from groups of formula -OCH₂CN, -OCH₂COR⁸ and -OCH₂CH₂OR⁹, where R⁸ is hydroxyl, (C₁-C₆)oxyalkyl, (C₁-C₆)oxyalkylphenyl, amino, (C₁-C₆)aminoalkyl, (C₁-C₆)aminodialkyl, (C₁-C₆)aminoalkylphenyl, an amino acid or derivative thereof; and R⁹ is (C₁-C₆)alkyl, (C₁-C₆)alkylphenyl, phenyl, substituted phenyl (C₁-C₆)alkylacyl or phenacyl;
- R₄ hydrogen or a (C₁-C₆)alkyl, or phenyl(C₁-C₆)alkyl group;
- R₅ hydrogen or methyl ;

12. The use as claimed in claim 1 of a compound forming part of the state of the art by virtue of the patent publication WO 92/22523 (Research Corporation Technologies) ("RCT"), namely a compound of formula (I)

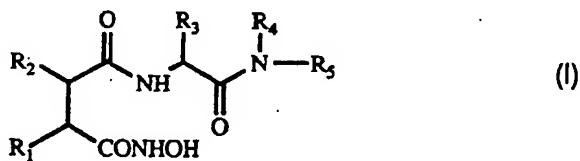


wherein the five substituents R₁ - R₅ may vary as follows:

- R₁ hydrogen, lower alkyl, aryl, or aryl lower alkyl;

- R₂ hydrogen, lower alkyl, aryl, or aryl lower alkyl;
- R₃ aryl lower alkyl, or heterocyclic lower alkyl, either being unsubstituted or mono- or di-substituted with fluoro, bromo, chloro, halo, nitro, carboxy, lower carbalkoxy, cyano, lower alkanoyl, trifluoromethyl lower alkyl, hydroxy, lower alkoxy, formyl, amino, lower alkyl amino, di-lower alkyl amino, mercapto, lower alkylthio, or mercapto lower alkyl;
- R₄ a group -XD wherein X is a bond, lower alkylene, or a group -(CAB)_m-C(O)-N(Z)-, or -(CAB)_m-CH₂O-, or -(CAB)_m-C(O)O- wherein A is hydrogen, methyl or ethyl, and B and Z are independently hydrogen or lower alkyl, and m is 1, 2 or 3 with the proviso that when X is a chemical bond then the group R₃ (see above for RCT definition of R₃) is not unsubstituted benzyl or benzyl monosubstituted with hydroxy or lower alkoxy, and the further proviso that when X is a group -(CAB)_m-C(O)-N(Z)- wherein m is 1 and A, B and Z are as defined above, then the group R₃ (see above for RCT definition of R₃) is not indole or imidazole or unsubstituted benzyl or benzyl substituted with hydroxy or lower alkoxy;
- R₅ hydrogen or lower alkyl;

13. The use as claimed in claim 1 of a compound forming part of the state of the art by virtue of the patent publication WO 93/09090 ("Yamanouchi"), namely a compound of formula (I)



wherein the five substituents R₁ - R₅ may vary as follows:

- R₁ lower alkyl which may be substituted by a substituent selected from mercapto, lower alkylthio, arylthio and lower acylthio;
- R₂ a group -Y-X-Z wherein Y is a single bond or lower alkylene, X is O or S,

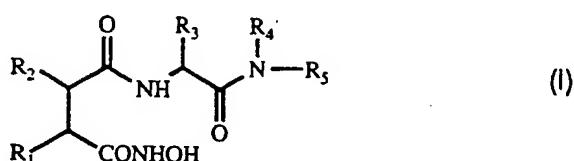
and Z is lower alkyl;

R₃ 4-(lower alkyl)phenylmethyl;

R₄ lower alkyl;

R₅ hydrogen;

14. The use as claimed in claim 1 of a compound forming part of the state of the art by virtue of the patent publication WO 93/09097 ("Sankyo"), namely a compound of formula (I)



wherein the five substituents R₁ - R₅ may vary as follows:

R₁ hydrogen;

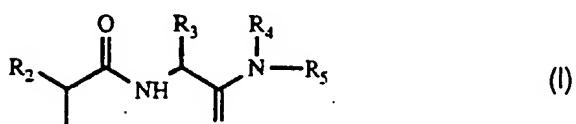
R₂ hydrogen, alkyl or aralkyl;

R₃ a group -CH₂-CH₂-CH₂-NH- where the right hand bond is formed with the N atom adjacent the C atom carrying R₃, thereby replacing the H atom shown on that C atom in formula (I);

R₄ hydrogen, alkyl, alkoxy, or (when R₅ is hydrogen) -CH(R⁶)COR⁷ where R⁶ is hydrogen or alkyl and R⁷ is alkyl, or -CH(R⁶)COOR⁸ where R⁶ is hydrogen or alkyl and R⁸ is alkyl, or -CH(R⁶)CONR⁹R¹⁰ where R⁹ and R¹⁰ are each alkyl, or R₄ taken together with R₅ and the nitrogen atom to which they are attached form a heterocyclic group;

R₅ hydrogen or alkyl;

15. The use as claimed in claim 1 of a compound of formula (I):



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wherein the five substituents R₁ - R₅ may vary as follows:

- R₁ hydrogen, C₁-C₆ alkyl, C₂-C₆ alkenyl, phenyl, substituted phenyl, phenyl(C₁-C₆ alkyl), heterocyclyl, or a group BSO_nA- wherein n is 0, 1 or 2 and B is hydrogen or a (C₁-C₆) alkyl, phenyl, substituted phenyl, heterocyclyl, C₁-C₆ acyl, phenacyl or substituted phenacyl group, and A represents C₁-C₆ alkyl; amino; protected amino; acylamino; OH; SH; C₁-C₆ alkoxy; C₁-C₆ alkylamino; C₁-C₆ alkylthio; aryl(C₁-C₆ alkyl); amino(C₁-C₆ alkyl); hydroxy(C₁-C₆ alkyl), mercapto(C₁-C₆ alkyl) or carboxy(C₁-C₆ alkyl) wherein the amino-, hydroxy-, mercapto- or carboxyl-group are optionally protected or the carboxyl- group amidated; lower alkyl substituted by maleimido, succinimido, naphthalimido, 2,3-dihydro-1,3-dioxo-1H-benz[d,e]isoquinol-2-yl, carbamoyl, mono(lower alkyl)carbamoyl, di(lower alkyl)carbamoyl, di(lower alkyl)amino, carboxy-lower alkanoylamino, pyrrolidino or morpholino;
- R₂ (C₁-C₆)alkyl; (C₂-C₆)alkenyl; phenyl(C₁-C₆)alkyl; cycloalkyl (C₁-C₆)alkyl; cycloalkenyl (C₁-C₆)alkyl; substituted amino; carboxyl; esterified carboxyl; or a group -Y-X-Z wherein Y is a single bond or (C₁-C₆)alkylene, X is O or S, and Z is (C₁-C₆)alkyl;
- R₃ C₁-C₆ alkyl; benzyl; benzyloxybenzyl; (C₁-C₆ alkoxy)benzyl; benzyloxy(C₁-C₆ alkyl); the characterising group of a natural α amino acid, which may be protected if functional groups are present, eg by acylation of amino groups and amidation of carboxyl groups; a group -[Alk]_nR⁶ where Alk is an C₁-C₆ alkyl or C₂-C₆ alkenyl group optionally interrupted by one or more -O-, or -S- atoms or -N(R⁷)-groups [where R⁷ is a hydrogen atom or a C₁-C₆ alkyl group], n is 0 or 1, and R⁶ is an optionally substituted cycloalkyl or cycloalkenyl group; a benzyl group with one or two substituents in the phenyl ring selected from hydrogen, halogen, cyano amino, amino (C₁-C₆)alkyl, amino di (C₁-C₆)alkyl, amino

(C₁-C₆)alkylacyl, aminophenacyl, amino(substituted)phenacyl, amino acid or derivative thereof, hydroxy, oxy (C₁-C₆)alkyl, oxyacyl, formyl, carboxylic acid, carboxamide, carboxy (C₁-C₆)alkylamide, carboxyphenylamide, carboxy (C₁-C₆)alkyl, hydroxy (C₁-C₆)alkyl, (C₁-C₆)alkyloxy (C₁-C₆)alkyl or acyloxy (C₁-C₆)alkyl, (C₁-C₆)alkylcarboxylic acid, or (C₁-C₆)alkylcarboxy (C₁-C₆)alkyl, amino(C₁-C₆)alkylacyl carboxylic acid or amino(C₁-C₆)alkylcarboxylate; or a benzyl group with one substituent in the phenyl ring selected from groups of formula - OCH₂CN, -OCH₂COR⁸ and -OCH₂CH₂OR⁹, where R⁸ is hydroxyl, (C₁-C₆)oxyalkyl, (C₁-C₆)oxyalkylphenyl, amino, (C₁-C₆)aminoalkyl, (C₁-C₆)aminodialkyl, (C₁-C₆)aminoalkylphenyl, an amino acid or derivative thereof; and R⁹ is (C₁-C₆)alkyl, (C₁-C₆)alkylphenyl, phenyl, substituted phenyl (C₁-C₆)alkylacyl or phenacyl; heterocyclic lower alkyl, either being unsubstituted or mono- or di-substituted with halo, nitro, carboxy, C₁-C₆ alkoxy, cyano, C₁-C₆ alkanoyl, trifluoromethyl C₁-C₆ alkyl, hydroxy, formyl, amino, C₁-C₆ alkyl amino, di-C₁-C₆ alkyl amino, mercapto, C₁-C₆ alkylthio, or mercapto C₁-C₆ alkyl; C₁-C₆ alkyl)phenylmethyl;

R₄ hydrogen, C₁-C₆ alkyl; a group -CHBD wherein B is H and D is H or C₁-C₆ alkyl, C₁-C₆ alkoxy-C₁-C₆ alkyl, di(C₁-C₆ alkoxy)methyl, carboxy, C₁-C₆ alkylcarbonyl, C₁-C₆ alkoxy carbonyl, arylmethoxycarbonyl, C₁-C₆ alkylaminocarbonyl or arylaminocarbonyl; or R₃ and B together form a group (CH₂)_n where n is an integer from 4-11; phenyl(C₁-C₂ alkyl), or trifluoromethylphenyl(C₁-C₂ alkyl); a group -(CH₂)_nA wherein n is an integer from 1 to 6 and A represents the group -NH₂, a substituted acyclic amine, a heterocyclic base, hydroxy, (C₁-C₆)alkoxy, (C₂-C₇)acyloxy, (C₁-C₆)alkylthio, phenylthio, (C₂-C₇)acylamino or an N-pyrrolidone group; an optionally substituted straight or branched alkyl group, optionally interrupted by one or more -O- or -S- atoms or -N(R⁷)- groups [where R⁷ is a hydrogen atom or a C₁-C₆ alkyl group], or aminocarbonyloxy groups; C₁-C₆ alkyl optionally substituted by aryl,

amino, protected amino, di(lower alkyl)amino, guanidino, carboxyl, protected carboxyl, carbamoyl; mono(lower alkyl)carbamoyl, di(lower alkyl)carbamoyl, di(lower alkyl)phosphinyl, dihydroxyphosphinyl, pyrrolidino, piperidino or morpholino;

R₅ hydrogen;

16. The use as claimed in claim 1 of a compound selected from the group consisting of:

2S-(4-methoxyphenylsulfanylmethyl)-3R-(2-phenyl-1S-methylcarbamoyl-ethylcarbamoyl)-5-methyl-hexanohydroxamic acid.

2S-(3-chlorophenylsulfanylmethyl)-3R-(2-phenyl-1S-methylcarbamoyl-ethylcarbamoyl)-5-methyl-hexanohydroxamic acid.

2S-(phenylsulfanylmethyl)-3R-(2-phenyl-1S-(pyrid-3-ylmethylcarbamoyl)-ethylcarbamoyl)-5-methyl-hexanohydroxamic acid.

2S-(3-methylphenylsulfanylmethyl)-3R-(2-phenyl-1S-methylcarbamoyl-ethylcarbamoyl)-5-methyl-hexanohydroxamic acid.

2S-(thien-2-ylsulphanylmethyl)-3R-(2-(4-carboxymethoxyphenyl)-1S-methylcarbamoyl-ethylcarbamoyl)-5-methyl-hexanohydroxamic acid.

2S-(thien-2-ylsulphanylmethyl)-3R-(2-phenyl-1S-(pyrid-3-ylmethylcarbamoyl)-ethylcarbamoyl)-5-methyl-hexanohydroxamic acid.

2S-(4-hydroxyphenylsulphanylmethyl)-3R-(2-phenyl-1S-(pyrid-3-ylmethylcarbamoyl)-ethylcarbamoyl)-5-methyl-hexanohydroxamic acid.

2S-(thien-2-ylsulfanylmethyl)-3R-(2-naph-2-yl-1S-methylcarbamoyl-ethylcarbamoyl)-5-methyl-hexanohydroxamic acid.

2S-(4-hydroxyphenylsulfanylmethyl)-3R-(2R-hydroxy-1S-methylcarbamoyl-

SUBSTITUTE SHEET

propylcarbamoyl)-5-methyl-hexanohydroxamic acid.

2S-(4-hydroxyphenylsulfanyl methyl)-3R-(5-acetamido-1S-methylcarbamoyl-pentylcarbamoyl)-5-methyl-hexanohydroxamic acid.

2S-(4-hydroxyphenylsulfanyl methyl)-3R-(3-[1,1-dimethylethoxycarbonyl]-1S-methylcarbamoyl-propylcarbamoyl)-5-methyl-hexanohydroxamic acid.

17. The use as claimed in claim 1 of a compound selected from the group consisting of:

2S-(thien-2-ylsulfonylmethyl)-3R-(2-phenyl-1S-methylcarbamoyl-ethylcarbamoyl)-5-methyl-hexanohydroxamic acid.

3S-(2-[4-acetamido-phenyl]-1S-methylcarbamoyl-ethylcarbamoyl)-5-methyl-hexanohydroxamic acid.

2S-(4-phthalimido-butyl)-3R-(3-methyl-1S-ethoxycarbonylmethylcarbamoyl-butylcarbamoyl)-5-methyl-hexanohydroxamic acid.

3R-(2-[4-methoxy-phenyl]-1S-methylcarbamoyl-ethylcarbamoyl)-2S,5-dimethyl-hexanohydroxamic acid.

3R-(2-phenyl-1S-[2-oxo-pyrolid-1-yl]propylcarbamoyl-ethylcarbamoyl)-5-methyl-hexanohydroxamic acid.

3R-(2-[4-methoxy-phenyl]-1S-methylcarbamoyl-ethylcarbamoyl)-5-methyl-hexanohydroxamic acid.

3R-(2-phenyl-1S-[pyrid-3-ylmethylcarbamoyl]-ethylcarbamoyl)-5-methyl-hexanohydroxamic acid.

3R-(2,2-dimethyl-1S-methylcarbamoyl-propylcarbamoyl)-5-methyl-hexanohydroxamic acid.

2S-(thien-2-ylsulphonylmethyl)-3R-(2-(4-carboxymethoxyphenyl)-1S-methylcarbamoyl-ethylcarbamoyl)-5-methyl-hexanohydroxamic acid.

18. A method of management (by which is meant treatment or prophylaxis) of diseases mediated by TNF in mammals, in particular in humans, which method comprises administering to the mammal an effective amount of a compound forming part of the state of the art by virtue of any of the patent publications listed in claim 1.
19. A method as claimed in claim 18 wherein the compound is as defined in any one of claims 2 to 17.
20. A use as claimed in any one of claims 1 to 17, or a method as claimed in claim 18 or claim 19 in which the disease mediated by TNF is inflammation, fever, cardiovascular disorder, haemorrhage, coagulation, acute phase response, cachexia, anorexia, acute infection, shock state, graft versus host reaction or autoimmune disease.
21. A use as claimed in any one of claims 1 to 17, or a method as claimed in claim 18 or claim 19 in which the disease is inhibition of the release of and the effects of TNF in septic shock, haemodynamic shock or sepsis syndrome, in post ischaemic reperfusion injury, in malaria, mycobacterial infection and meningitis, in psoriasis, in cancer, in cachexia, in fibrotic disease, in congestive heart failure, in graft rejection, in rheumatoid arthritis, in radiation damage and toxicity following administration of immunosuppressive monoclonal antibodies such as OKT3 or CAMPATH-1, in hyperoxic alveolar injury, in autoimmune disease for example AIDS and multiple sclerosis, and in any disease state where TNF is a mediator of host injury.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 93/02331

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 A61K31/16

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 5 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US,A,4 599 361 (FUKAMI ET AL) 8 July 1986 cited in the application ---	1-21
Y	EP,A,0 236 872 (HOFFMAN-LA ROCHE) 16 September 1987 cited in the application ---	1-21
Y	EP,A,0 274 453 (LAB ROGER BELLON) 13 July 1988 cited in the application ---	1-21
Y	WO,A,90 05716 (BRITISH BIOTECHNOLOGY LTD) 31 May 1990 cited in the application ---	1-21
Y	WO,A,90 05719 (BRITISH BIOTECHNOLOGY LTD) 31 May 1990 cited in the application ---	1-21
	-/-	

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

* Special categories of cited documents :

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INTERNATIONAL SEARCH REPORT

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PCT/GB 93/02331

C(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO,A,91 02716 (BRITISH BIOTECHNOLOGY LTD) 7 March 1991 cited in the application ---	1-21
Y	EP,A,0 489 577 (CELLTECH LTD) 10 June 1992 cited in the application ---	1-21
Y	EP,A,0 489 579 (CELLTECH LTD) 10 June 1992 cited in the application ---	1-21
Y	EP,A,0 497 192 (HOFFMANLA ROCHE) 5 August 1992 cited in the application ---	1-21
Y	WO,A,92 13831 (BRITISH BIOTECHNOLOGY LTD) 20 August 1992 cited in the application ---	1-21
P,Y	WO,A,92 22523 (RESEARCH CORPORATION TECHNOLOGIES) 23 December 1992 cited in the application ---	1-21
P,Y	WO,A,93 09090 (YAMANOUCHI CO. LTD) 13 May 1993 cited in the application ---	1-21
P,Y	WO,A,93 09097 (SANKYO) 13 May 1993 cited in the application ---	1-21
Y	EP,A,0 486 809 (FARMITALIA CARLO ERBA) 27 May 1992 -----	1-21

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 93/02331

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
US-A-4599361	08-07-86	AU-B-	588362	14-09-89
		AU-A-	6240886	12-03-87
		EP-A, B	0214639	18-03-87
		JP-A-	62103052	13-05-87
		US-A-	4743587	10-05-88
EP-A-0236872	16-09-87	AU-B-	588437	14-09-89
		AU-A-	6990287	17-09-87
		CA-A-	1314655	16-03-93
		DE-A-	3782751	07-01-93
		JP-A-	62230757	09-10-87
		US-A-	4996358	26-02-91
EP-A-0274453	13-07-88	FR-A-	2609289	08-07-88
		JP-A-	63258449	25-10-88
		US-A-	4918105	17-04-90
WO-A-9005716	31-05-90	AU-B-	641629	30-09-93
		AU-A-	4746890	12-06-90
		EP-A-	0445206	11-09-91
		JP-T-	4503057	04-06-92
WO-A-9005719	31-05-90	AU-B-	644064	02-12-93
		AU-A-	4800390	12-06-90
		EP-A-	0446267	18-09-91
		JP-T-	4502008	09-04-92
		US-A-	5240958	31-08-93
WO-A-9102716	07-03-91	AU-B-	639706	05-08-93
		AU-A-	6045490	03-04-91
		EP-A-	0489032	10-06-92
		JP-T-	5501864	08-04-93
EP-A-0489577	10-06-92	AU-A-	9017391	25-06-92
		AU-A-	9023391	25-06-92
		EP-A-	0489579	10-06-92
		WO-A-	9209564	11-06-92
		WO-A-	9209565	11-06-92
		GB-A-	2255339	04-11-92
		GB-A-	2255340	04-11-92

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 93/02331

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-0489577		HU-A-	61973	29-03-93
		JP-T-	5503719	17-06-93
		JP-T-	5503720	17-06-93
EP-A-0489579	10-06-92	AU-A-	9017391	25-06-92
		AU-A-	9023391	25-06-92
		EP-A-	0489577	10-06-92
		WO-A-	9209564	11-06-92
		WO-A-	9209565	11-06-92
		GB-A-	2255339	04-11-92
		GB-A-	2255340	04-11-92
		HU-A-	61973	29-03-93
		JP-T-	5503719	17-06-93
		JP-T-	5503720	17-06-93
EP-A-0497192	05-08-92	AU-A-	1025792	06-08-92
		JP-A-	4352757	07-12-92
WO-A-9213831	20-08-92	AU-A-	1194492	07-09-92
		EP-A-	0498665	12-08-92
WO-A-9222523	23-12-92	AU-A-	2228292	12-01-93
WO-A-9309090	13-05-93	JP-A-	5125029	21-05-93
		AU-A-	2799792	07-06-93
WO-A-9309097	13-05-93	AU-A-	2799392	07-06-93
		JP-A-	5194414	03-08-93
		JP-A-	5194415	03-08-93
EP-A-0486809	27-05-92	JP-A-	4283515	08-10-92